1-1-2016

Coloration in the polymorphic frog Oophaga pumilio associates with level of aggressiveness in intraspecific and interspecific behavioral interactions

Sandra P. Galeano
Louisiana State University

Kyle E. Harms
Louisiana State University

Recommended Citation

Galeano, S., & Harms, K. (2016). Coloration in the polymorphic frog Oophaga pumilio associates with level of aggressiveness in intraspecific and interspecific behavioral interactions. *Behavioral Ecology and Sociobiology, 70* (1), 83-97. https://doi.org/10.1007/s00265-015-2027-5

Behav Ecol Sociobiol (2016) 70:83–97
DOI 10.1007/s00265-015-2027-5

ORIGINAL ARTICLE

Coloration in the polymorphic frog *Oophaga pumilio* associates with level of aggressiveness in intraspecific and interspecific behavioral interactions

Sandra P. Galeano[1] · **Kyle E. Harms**[1]

Received: 9 August 2015 / Revised: 6 October 2015 / Accepted: 7 October 2015 / Published online: 4 November 2015

Abstract Intraspecific morphological variation may correspond to behavioral variation that helps determine the nature of species interactions. Color variation among populations of variably toxic organisms has been shown to associate with alternative anti-predator behaviors. However, the effects of these alternative behavioral tendencies on the outcomes of interspecific interactions other than predator–prey remain largely unexplored. We investigated how coloration and body size variation in *Oophaga pumilio*, one of the most phenotypically diverse amphibians known, associated with territorial aggressiveness and how this association influenced the outcome of agonistic male–male interactions with conspecifics and heterospecifics of two sympatric species (*Andinobates claudiae* and *Phyllobates lugubris*). Irrespective of body size, resident frogs from more conspicuous, red-colored *O. pumilio* populations responded to same-morph conspecifics and *P. lugubris* more quickly and exhibited more aggressive behaviors and more energetically expensive behaviors than resident frogs from green populations under these same treatments. Furthermore, red-colored resident frogs dominated most of the interactions in which they were involved, whereas green residents dominated only a few of the interactions, despite their status as residents. Because conspecific and heterospecific intruders did not behave more aggressively toward red resident frogs, aggressiveness of red residents does not appear to be a response to higher aggression being directed toward them. These results suggest that coloration in *O. pumilio* is a good indicator of aggressiveness that associates with the outcome of intraspecific and some interspecific behavioral male–male interactions, providing support for a positive association among anti-predator traits, agonistic behavior, and dominance in both intraspecific and interspecific, intraguild interactions.

Keywords Agonistic interactions · Aposematism · Body size · Conspicuousness · Dominance · Dendrobatidae

Introduction

Individuals within species differ in multiple morphological traits that may associate with behaviors that can influence the strength of intraspecific and interspecific agonistic interactions. Intrapopulation morphological variation is associated with variation in alternative reproductive tactics and aggression levels in several taxa. Body size, for example, is often a good predictor of aggressiveness and dominance in agonistic intraspecific interactions (reviewed in Hsu et al. 2006). Similarly, presumed signaling traits, such as coloration, may function as good indicators of aggression and dominance in conspecific (Sinervo and Lively 1996; Pryke and Griffith 2006; Healey et al. 2007; Dijkstra et al. 2009; Bastiaans et al. 2013) and some heterospecific encounters (Dijkstra et al. 2005; Anderson and Grether 2010; Lehtonen et al. 2010).

Coloration is expected to correlate with behavior to increase the effectiveness of anti-predator strategies.

Communicated by K. Summers

Electronic supplementary material The online version of this article (doi:10.1007/s00265-015-2027-5) contains supplementary material, which is available to authorized users.

✉ Sandra P. Galeano
sgalea1@tigers.lsu.edu

[1] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

Behavioral traits that increase exposure, for example, should be favored in conspicuous organisms because the exaggeration of both traits may increase the effectiveness of signals. This kind of *phenotypic integration* (i.e., the correlation of multiple phenotypic traits reflecting genetic, developmental, or functional interactions) may evolve when some phenotypic combinations of different traits are favored by natural selection (Pigliucci 2003). In multiple taxa of toxic prey, enhanced protection from predators via aposematism may counterbalance the selective disadvantage of behaviors that would otherwise increase detection (e.g., foraging and movement; Grant 2007; Speed et al. 2010; Pröhl and Ostrowski 2011). The rate at which these behaviors occur influences conspicuousness and simultaneously influences escape behavior. As a result, behaviors that enhance detection are expected to be favored in aposematic organisms because they increase the effectiveness of signals, whereas the opposite is expected for non-toxic, cryptic prey (Grant 2007; Speed et al. 2010).

Several examples of associations between coloration conspicuousness and behavior come from comparisons among species (Stamp and Wilkens 1993; Bernays and Singer 2002; Merilaita and Tullberg 2005). However, more recent studies have examined this association among individuals of single conspicuous populations (Bastiaans et al. 2013; Rojas et al. 2014; Crothers and Cummings 2015) or among morphologically distinct populations of a single species (Mochida 2009; Pröhl and Ostrowski 2011; Rudh et al. 2013; Willink et al. 2013). For example, populations with more conspicuous ventral patches in the salamander *Cynops pyrrhogaster* performed the "unken reflex" (an anti-predator behavior that enhances the effectiveness of the aposematic coloration) more frequently than salamanders with less conspicuous patches (Mochida 2009). Similarly, individuals from more conspicuous, red populations of the poison frogs *Oophaga pumilio* and *O. granulifera* were more active and spent more time foraging than frogs from green populations (Pröhl and Ostrowski 2011; Willink et al. 2013). Brightness (a conspicuousness-related trait that increases predator learning; Prudic et al. 2007) associated with aggressive behavior in one of the conspicuous populations of *O. pumilio*. Brighter males were more willing to initiate aggressive interactions with conspecifics by calling more quickly than their duller counterparts (Crothers and Cummings 2015), eliciting more calls to bright rivals and exhibiting lower advertisement call pulse rates (Crothers et al. 2011). These studies suggest that color variation among populations of toxic organisms can associate with alternative behavioral strategies that include alternative anti-predator behavior. However, none of these studies have examined the effects of these strategies on the outcomes of interspecific interactions other than those between predator and prey. In this study, we take a different approach, by examining the effects of these strategies on interspecific, intraguild agonistic interactions.

Agonistic interactions among animals (i.e., interactions associated with conflict) are often mediated by aggressive behavior. Aggression among conspecifics affects access to resources and reproductive opportunities, whereas heterospecific aggression usually relates to resource use (Peiman and Robinson 2010). Heterospecific aggression is widespread in nature and is often as costly as intraspecific aggression (Peiman and Robinson 2010; Grether et al. 2013). Furthermore, heterospecific aggression has the potential to influence the abundance and distribution of species if more aggressive species obtain better access to resources or to more valuable resources by securing better territories, while excluding less aggressive species (Robinson and Terborgh 1995; Duckworth and Badyaev 2007). Despite its prevalence and potential effect on community structure, heterospecific aggression has not received the same attention as aggression among conspecifics (Peiman and Robinson 2010) or other interspecific interactions in ecological studies (Grether et al. 2013).

We used the territorial and extremely phenotypically diverse poison frog *O. pumilio* to examine the association of coloration and body size with aggressiveness, and its effects on the outcomes of agonistic interactions with conspecifics of the same morph and heterospecifics. *O. pumilio* is relatively monomorphic in color in Nicaragua and Costa Rica but exhibits extensive morphological variation in coloration and size in the Caribbean coast of northwestern Panama, including the Bocas del Toro Archipelago (Daly and Myers 1967). Frogs from different islands in this archipelago exhibit either conspicuous, presumably aposematic colors such as red or orange, or dull, presumably cryptic colors such as green (Wang and Shaffer 2008; Maan and Cummings 2012). However, one population on the northwest tip of one island (Isla Bastimentos; Wang and Shaffer 2008; Richards-Zawacki and Cummings 2010) is polymorphic in color. At least 15 phenotypes occur in the archipelago, covering almost the entire visible spectrum range (Daly and Myers 1967; Maan and Cummings 2009). *O. pumilio* also vary in size among islands, exhibiting a body size reduction toward the southeast of the archipelago (Pröhl et al. 2007). This body size gradient does not seem to coincide with a coloration gradient, as large-bodied and small-bodied populations are represented by both red and green morphs.

Previous research indicates that sexual selection plays an important role in driving color divergence for *O. pumilio* (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2009; Richards-Zawacki and Cummings 2010), possibly following geographic isolation and drift (Rudh et al. 2007; Brown et al. 2010), expansion and isolation (Gehara et al. 2013), or interacting with drift (Tazzyman and Iwasa 2010). Natural selection by predators may also influence color divergence (Crothers and Cummings 2013) and may interact with sexual selection (Rudh et al. 2011; Maan

and Cummings 2012) to drive this divergence. Cumulative evidence also suggests that more conspicuous morphs are presumably aposematic in contrast to less conspicuous morphs (Siddiqi et al. 2004; Maan and Cummings 2008, 2012; Hegna et al. 2013). Conspicuousness of frog coloration correlated with frog toxicity on some islands of the archipelago (Maan and Cummings 2012; but see Daly and Myers 1967), suggesting that coloration may be an honest signal of unprofitability to predators. Vision models revealed that colors exhibited by *O. pumilio* can be easily detected by both conspecifics and model predators (Siddiqi et al. 2004; Maan and Cummings 2012). In addition, red clay models suffered lower attack rates (interpreted as predation) than green models on an island where green was the resident morph (Hegna et al. 2013). Understanding the causes of variation in coloration in *O. pumilio* has been the focus of studies in this region for decades, but little attention has been directed toward the potential ecological implications that both coloration and body size variation in this species have on species interactions other than those between frogs and their predators.

In this study, we examined how intraspecific variation in coloration and body size in *O. pumilio* associates with territorial aggressiveness and how this association influences agonistic interactions with conspecifics of the same morph and heterospecifics. We focused on the chromatic component of coloration instead of the brightness component, which has been the focus of recent studies in this species (see above). We hypothesized that coloration would be a good indicator of aggressive behavior and the outcome of agonistic interactions in *O. pumilio* independent of body size, as a result of the enhanced protection from predators that would allow more conspicuous, presumably aposematic frogs to exhibit bolder behaviors that enhance detection, than less conspicuous, presumably cryptic frogs. We predicted that frogs from more conspicuous, red populations of *O. pumilio*—as compared to frogs from less conspicuous, green populations—would (1) exhibit enhanced aggression toward conspecifics of the same morph and heterospecifics and (2) dominate most intraspecific and interspecific encounters. We built our predictions on the underlying assumption that frogs from more conspicuous, red populations exhibit aposematic strategies, in contrast to frogs from less conspicuous, green populations. This assumption is based on cumulative evidence from previous studies (Siddiqi et al. 2004; Maan and Cummings 2008, 2012; Pröhl and Ostrowski 2011; Hegna et al. 2013). Regardless of whether or not the assumption of aposematism is correct for the red morphs vs. green morphs, finding associations between coloration and heterospecific aggressiveness would provide evidence for a connection between population-specific traits and the strength of interspecific, intraguild behavioral interactions.

Methods

We conducted a paired resident/intruder encounter experiment at the Bocas del Toro Research Station, Smithsonian Tropical Research Institute, Panama, in 2012. In all trials of this experiment, an individual (intruder) was placed in the territory of another individual (resident) in a manner that permitted non-lethal conflict. None of the frogs were injured during the experiment.

Frog species

We included three species from the Bocas del Toro Archipelago: *O. pumilio*, *Phyllobates lugubris*, and *Andinobates claudiae* (Dendrobatidae). These are small poison frogs that use similar habitats for foraging, calling, and breeding in the forested habitats on some islands of the Bocas del Toro Archipelago and nearby mainland. Frogs of these species can be found in primary and secondary forests, foraging and laying their eggs on the leaf litter and transporting their larvae to phytotelmata, including *Heliconia* bracts, bromeliad axils, tree holes, and other small pools on or above the forest floor (Savage 2002). A few studies suggest that competition for resources potentially occurs among the species studied. *P. lugubris* was suggested to compete for food sources with *O. pumilio* (Baugh and Forester 1994). Diet overlap between these two species is likely a result of their preference for formicids (Lieberman 1986) and their similar body size, which associated with prey size within frog specialist guilds in previous studies (Toft 1980). Also, calls of *P. lugubris* are likely potent acoustic maskers for *O. pumilio* calls. The two species' calls closely resemble one another, and *O. pumilio* lowered the dominant frequency of its calls following playbacks of *P. lugubris* (Wong et al. 2009). This suggests that acoustic communication interference could occur between these two species. *O. pumilio* and *A. claudiae* deposit their larvae in water-filled leaf axils (Lötters et al. 2007); limitation by this shared resource could result in competition. Competition among conspecifics for limited bromeliad availability was observed in *O. pumilio* (Donnelly 1989), and phytotelmata have been suggested to be limited resources for poison frogs in some areas (Donnelly 1989; Heying 2004).

O. pumilio is a territorial species, with populations from different islands generally exhibiting either conspicuous, presumably aposematic colors or dull, presumably cryptic colors (Wang and Shaffer 2008; Maan and Cummings 2012). This species also exhibits remarkable body size variation in the archipelago, with large-bodied populations (males, ~19 mm in length) found on northern islands and small-bodied populations (males, ~16.5 mm in length) found on southern islands (Pröhl et al. 2007). *P. lugubris* can have a similar size or be larger than *O. pumilio* (males, up to 21 mm), whereas

A. claudiae is smaller (males, 13.4 mm in length; Lötters et al. 2007).

Prior to conducting the experiment, we collected 25 males of *O. pumilio* from each of four islands, totaling 100 individuals. The islands on which we sampled *O. pumilio* were based on the morph present: two islands have more conspicuous, red *O. pumilio* populations than the other two islands which have less conspicuous, green populations. Red and green color morphs were selected because they are predominant in the species (Pröhl and Ostrowski 2011). Each island of each color morph had either a large-bodied or a small-bodied population of *O. pumilio* (Table 1). We also collected 40 total males of *P. lugubris* and 40 total males of *A. claudiae* from a combination of these same islands and two additional islands (Table 1). Collection of *P. lugubris* and *A. claudiae* on these additional islands was required because we found no males at the time of our experiment on some islands where we collected *O. pumilio*. Despite this, we consider that our interspecific tests are valid because the three species included in this study co-occur broadly on several islands of the archipelago and nearby mainland, making these tests informative. Only males of *P. lugubris* and *A. claudiae* found calling in the field were captured. After capture, we measured the body size (estimated as the snout vent length (SVL)) of all frogs to the nearest 0.01 mm using a caliper.

Frogs were allowed to form territories for at least eight days in separate transparent terraria (40×20×10 cm). One week was described as sufficient time for *O. pumilio* to form territorial bounds under laboratory conditions (Forester et al. 1993; Baugh and Forester 1994). Terraria were covered with black plastic on one side to keep frogs from seeing each other while in captivity. Frogs could hear neighbors calling, although we do not think this affected our experiment because *O. pumilio* does not respond differentially to calls of neighbors and strangers (Bee 2003). To each terrarium, we added one bromeliad plant, one piece of wood, and 20 g of leaf litter. Similarly sized pieces of wood and bromeliads were used. Frogs were fed daily with vitamin-dusted ants, fruit flies, and termites, and water was provided ad libitum. Terraria were misted daily. Each *O. pumilio* male was assigned then to either play the role of resident or intruder during the experiment, whereas all *P. lugubris* and *A. claudiae* were assigned the role of intruders.

Experimental procedure

To conduct the resident/intruder encounter experiment, we challenged one *O. pumilio* resident from a red and small-bodied population, a red and large-bodied population, a green and small-bodied population, or a green and large-bodied population with (a) one similarly sized conspecific of the same morph (referred to as the intraspecific treatment), (b) one *P. lugubris* (referred to as the interspecific *P. lugubris* treatment), (c) one *A. claudiae* (referred to as the interspecific *A. claudiae* treatment), and (d) one surrogate frog model constructed using non-toxic clay of the same color as the resident (surrogate). Intraspecific encounters served as baselines to identify aggressive behaviors associated with *O. pumilio*

Table 1 Islands of the Bocas del Toro Archipelago in which frogs of *Oophaga pumilio*, *Phyllobates lugubris*, and *Andinobates claudiae* were collected

Island	*O. pumilio* coloration	*O. pumilio* Mean body size (size category)	*P. lugubris* Mean body size	*A. claudiae* Mean body size
Colon	Green	19.67±0.39 *n*=25 (large)	19.05±1.51 *n*=10	13.30±0.35 *n*=10
Bastimentos	Red	20.00±0.45 *n*=25 (large)	–[a]	13.26±0.36 *n*=10
Cayo de Agua	Green	17.23±0.55 *n*=25 (small)	19.12±1.55 *n*=10	13.25±0.29 *n*=10
Solarte (South)	Red	17.00±0.35 *n*=25 (small)	18.80±1.00 *n*=10	–
Popa[b]	Green		19.03±1.38 *n*=5	13.33±0.35 *n*=10
San Cristobal[b]	Red		18.92±0.45 *n*=5	

Dashes represent islands where males of a species were not found. The superscript next to a dash indicates that museum collections exist for the species. Note that we found males of the three species on only two islands. Therefore, we collected *P. lugubris* and *A. claudiae* males from two additional islands (superscript letter b). The coloration and size category of *O. pumilio* populations of each island surveyed is shown. The mean body size, standard deviation, and sample size of each species collected are provided

[a] These specimens were located trough VertNet (http://vertnet.org): Biodiversity Institute, University of Kansas (KUH 94831–94835); National Museum of Natural History, Smithsonian Institution, Amphibians & Reptiles (USNM 297854.6151782)

coloration that also could be observed in heterospecific encounters. Therefore, we considered it more relevant to include a conspecific same-morph treatment instead of a conspecific different-morph treatment, since our main focus was to examine the association between coloration and heterospecific agonistic interactions. Surrogates allowed us to differentiate between aggressive behaviors being directed toward a live intruder vs. an inanimate new object placed in a resident's territory. Even though the surrogate treatment does not allow us to identify if residents perceived models as frogs or as inanimate objects, they provide an indication of whether residents automatically respond to a novel object added to their territories or not. Each *O. pumilio* resident was paired separately with one individual from the above treatments, so that each resident experienced four separate trials (Fig. 1). Trials were conducted in random order. To do so, first, we randomly selected the island to be surveyed in each morning and each afternoon of the experiment. Second, we randomly selected the treatment that each resident to be tested that morning or afternoon would receive. This allowed us to use each frog in trials only once a day and also test frogs from each island on different days, minimizing possible winner–loser effects. Frogs of *P. lugubris* and *A. claudiae* collected from different islands were randomly paired with *O. pumilio* residents of each morphotype. Fifteen intruders were used twice during the experiment.

Before starting a trial, we covered two additional sides of the resident terrarium using black plastic to avoid disturbing the frogs. We then placed a male frog (intruder) in the tank of another male frog (resident). This was done by taking each individual (resident and intruder) out of its own terrarium and then simultaneously placing them next to each other at the center of the resident's terrarium and covering them with small individual plastic petri dishes. Removing and introducing both frogs in the same way allowed us to control for the effect of handling on the experimental outcome (Baugh and Forester 1994). We then covered the top of the terrarium using a sheet of transparent acrylic. Frogs were allowed to acclimate under petri dishes for 1 min before a trial and were allowed to see each other. Petri dishes were lifted using a string from outside of the terrarium, and frogs were allowed to interact for 15 min. Behaviors exhibited by both contestants were recorded during the trial and also videotaped with a Sony DCR-HC38 video camera from above the terrarium. Resident and intruder conspecifics were identified by their individual marks registered before starting a trial. We did not record data blind because our study involved focal individuals in the terraria.

We collected data for 10 to 13 trials for each treatment, totaling 196 (44 additional trials were discarded because frogs climbed onto the petri dishes and performed none of the behaviors we quantified). Trials were conducted between the months of November and December from 0900 to 1700 hours. The experiment was conducted in a dark room, where three 75 W UV lights and one 25 W halogen light, filtered by two green–blue filters (Lee 728+Cyan-Gel 4315) were used to illuminate the resident's terrarium, mimicking light conditions on the forest floor (Maan and Cummings 2008). Each frog was released at its place of capture after the experiment.

Measures of male aggression and contest outcome

The displays of agonistic behaviors by residents and intruders were quantified based on observations made during the trial

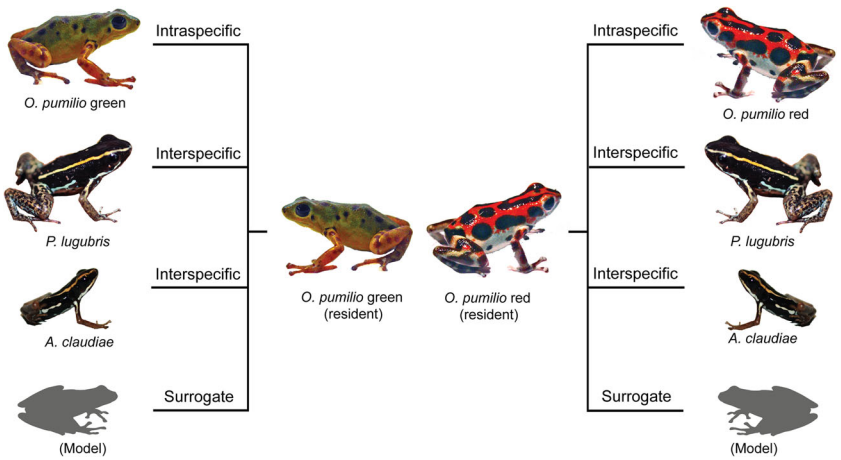

Fig. 1 Treatments included in the resident–intruder experiment. *Oophaga pumilio* residents from red and green populations were paired with one similarly sized conspecific of the same morph (intraspecific treatment), one *Phyllobates lugubris* (interspecific *P. lugubris* treatment), one *Andinobates claudiae* (interspecific *A. claudiae* treatment), and one surrogate frog constructed using non-toxic clay to resemble the same morph (surrogate). Each resident was paired separately with one individual from each treatment. Red and green residents from large-bodied populations are represented. Red and green residents from small-bodied populations were subjected to the same treatments (not shown)

and analysis of video recordings. We quantified 13 agonistic behaviors total (Table 2). Twelve of these behaviors were previously reported for *O. pumilio* (Baugh and Forester 1994; Hödl and Amézquita 2001). One additional agonistic behavior was identified for *O. pumilio*, whereas six behaviors were identified for *P. lugubris* and four for *A. claudiae* (Table 2).

We estimated three metrics to assess the level of aggression of resident and intruder frogs, and one metric to estimate contest outcome. First, we estimated an aggression score (*sensu* Sacchi et al. 2009), calculated as the number of aggressive behaviors minus the number of submissive behaviors displayed by an individual during the contest. Positive values represented predominance of aggressive behaviors, and negative values represented predominance of submissive behaviors. Second, we estimated an index of aggression (*sensu* Baugh and Forester 1994), based on a ranked evaluation of energy expenditure of each agonistic behavior (i.e., aggressive and submissive behaviors), previously published for *O. pumilio* (Baugh and Forester 1994), and estimated for the other two species based on observations in a pilot experiment (Table 2). This index assigns submissive behaviors negative values to subtract their contributions from the overall measure of aggressiveness. Ranked values varied from −1 to 4, with behaviors judged to be more energetically expensive receiving a higher value than less costly ones. Values for all behaviors exhibited by an individual in a trial were summed in order to obtain the index of aggression. Third, we estimated the latency to respond, measured as the time to first aggression by an individual. These three metrics represent different aspects of aggressiveness. Estimating both aggression score and index of aggression permits differentiation among individuals that (a) invest little in agonistic encounters (displaying a few aggressive behaviors that demand low energetic expenditure), (b) invest more (displaying numerous aggressive behaviors that demand high energetic expenditure), and (c) display either numerous aggressive behaviors that demand low energetic expenditure or vice versa. Estimating latency provides information on the willingness of an individual to take part in an agonistic encounter. Fourth, we assessed the contest outcome by estimating a contest score, calculated as the difference between the aggression scores of a resident and an intruder in a trial (Sacchi et al. 2009). Higher, positive contest score values represented dominance of the resident, whereas negative values represented dominance of the intruder.

Data analysis

We conducted separate generalized linear mixed model analyses to assess the influence of coloration, body size, and treatment on aggression score, index of aggression, latency, and contest score. We included coloration, body size, and treatment as fixed effects and males nested within coloration and body size as random effects. We used models with skewed *t* distribution error structure and the *identity* link (a canonical link for the normal distribution) to accommodate positive and

Table 2 Description of agonistic behaviors displayed by frogs of *Oophaga pumilio*, *Phyllobates lugubris*, and *Andinobates claudiae*. The description of behaviors follows Baugh and Forester (1994) and Hödl and Amézquita (2001)

Behavior	Description	Points	*O.pumilio*	*P.lugubris*	*A.claudiae*
Calling[a]	Vocalizing	1	X		
Pushups[a]	Elevating and lowering the anterior part of the body by extending the forelegs	1	X		
Body raising[b]	Elevating the whole body by extending all the four legs	1	X		X
Limb shaking[b]	Rapidly moving a limb up and down	1	X	X	
Tracking[a]	Orienting the body in the direction of opponent while in a static position or moving forward	1	X	X	X
Charge and veer[a]	Running toward opponent and moving away without contact	2	X	X	
Charge and contact[a]	Running toward opponent and initiating physical contact	3	X		
Grappling[a]	Seizing the opponent's limb(s)	3	X		
Pinning[a]	Pressing opponent to the ground	3	X		
Chase[a]	One frog pursues an encounter, while the other frog retreats	4	X	X	
Escape[a]	Escaping in response to aggression	−1	X	X	X
Statue[a]	Freezing in one position in response to aggression	−1	X	X	X
Body lowering	Lowering the body, pressing it against the substrate	−1	X		

The number of points assigned to each behavior is based on a ranked evaluation of energy expenditure through aggression published for *O. pumilio* by Baugh and Forester (1994) and by extensive observations in a pilot experiment. Xs represent behaviors observed for each species in this study. Superscripts denote behaviors previously reported for *Oophaga pumilio*

[a] Baugh and Forester (1994)

[b] Hödl and Amézquita (2001)

negative values in heavier tailed distributions. We ran mixed models, both adjusting the degrees of freedom (using the Kenward–Roger method, which inflates the variance/covariance matrix of fixed and random effects and then performs a Satterthwaite denominator degrees of freedom calculation to produce a more accurate F test for small samples; Kenward and Roger 1997), and without the adjustment. Because the outcome did not differ between these two methods, we only present results for the latter. We used Tukey's post hoc tests to determine where differences occurred among groups. We evaluated the magnitude of differences between groups based on effect sizes using Cohen's d and its 95 % confidence intervals, interpreted as small (d=0.20), moderate (d=0.50), and large (d=0.80) (Cohen 1988). We considered body size as a categorical variable as a result of the non-overlapping bimodal nature of the data for large-bodied and small-bodied populations of both green and red color morphs (Electronic supplementary material Fig. S1). Encounters in which none of the males responded aggressively were discarded for latency analyses (n=35). We did not include values of these encounters as extreme latency, because this would necessarily make those males' estimates false, as it is not possible to predict whether they would have responded either soon after or much later after the trials ended. We used a crossover effects model to test for treatment carryover effects that could represent the influence of prior fighting experience of residents on our experiment (winner–loser effects, Hsu et al. 2006). This test assesses whether performance by an individual exposed to a second treatment is affected by individual performance in a first treatment. However, no carryover effects were found.

Coloration and body size are not randomly or uniformly distributed in space in islands of the Bocas del Toro Archipelago (i.e., each color–size combination occurs on different islands). As a result, body size and coloration are not independent variables in our study and including both as independent fixed effects in the model could influence our results. Therefore, we ran two groups of new analyses, one excluding body size from the model and the other excluding coloration. By independently excluding each variable, we evaluated if our model was affected by including both variables as fixed effects in the initial analyses. Results between these two groups of analyses and initial analyses did not differ. We present results from analyses that included both coloration and body size here, due to the documented influence of both traits in the development and outcome of agonistic interactions. Main results of analyses excluding each variable are shown in Electronic supplementary material Tables S1 and S2.

We conducted separate generalized linear mixed model analyses to assess the influence of coloration, body size, and treatment on intruder's aggression score and index of aggression, following the initial analyses procedure. Even though residents and intruders likely influenced each other's behavior in our experiment, analyzing them independently allowed us to test whether higher levels of aggressiveness in red residents could result from higher aggression being directed toward them. We conducted all analyses using the PROC GLIMMIX procedure (an extension of Proc Mixed; SAS Institute I 2003).

Results

Aggressive interactions between *O. pumilio* and a same-morph conspecific occurred in 98 % of trials, in 85 % of trials when the species was paired with *P. lugubris*, and in 64 % of trials when paired with *A. claudiae*. Frogs did not respond aggressively to surrogates in any of the trials. Agonistic behaviors such as chasing, calling, and tracking were commonly observed in both intraspecific and interspecific interactions.

Resident agonistic behavior

Coloration interacted with treatment to explain the number of aggressive behaviors (aggression score) displayed by *O. pumilio* residents (color: $F_{1,57}$=13.99, p<0.001; treatment: $F_{3,124}$=29.09, p<0.001; color×treatment: $F_{3,124}$=6.87, p<0.001). Tukey's post hoc tests and Cohen's d effect sizes revealed that red residents paired with a same-morph conspecific exhibited more aggressive behaviors than green residents under this treatment or when paired with *P. lugubris* (Table 3, Fig. 2a). The aggression score of *O. pumilio* was significantly larger than zero in these treatments (Tukey's post hoc test: red intraspecific, p<0.001; green intraspecific, p<0.001; red interspecific *P. lugubris*, p<0.001; green interspecific *P. lugubris*, p<0.01; Fig. 2a), suggesting that *O pumilio* residents exhibited more aggressive than submissive behaviors when paired with a same-morph conspecific and *P. lugubris*. In contrast, red and green residents did not differ in their aggression scores when paired with *A. claudiae* or a surrogate (Table 3, Fig. 2a). Frogs from red large-bodied and red small-bodied populations exhibited similar aggression scores, as did frogs from green large-bodied and green small-bodied populations (Electronic supplementary material Table S3).

Coloration and treatment also interacted to explain the costs of aggressive behaviors (index of aggression) displayed by *O. pumilio* residents (color: $F_{1,57}$=14.52, p<0.001; treatment: $F_{3,124}$=25.20, p<0.001; color×treatment: $F_{3,124}$=5.84, p<0.001). Tukey's post hoc tests and Cohen's d effect sizes showed that red resident frogs exhibited more energetically expensive behaviors than green residents when paired with a same-morph conspecific or with *P. lugubris* (Table 3, Fig. 2b). However, no differences were found in the costs of behaviors displayed between red and green residents when paired with *A. claudiae* or a surrogate (Table 3, Fig. 2b). Frogs from red large-bodied and red small-bodied populations exhibited

Table 3 *p* values of Tukey's post hoc test and Cohen's *d* effect sizes for comparisons of the aggression score, index of aggression, and latency of green and red *O. pumilio* residents subjected to four experimental treatments

	Red vs. green			
	Intraspecific	Interspecific *P. lugubris*	Interspecific *A. claudiae*	Surrogate
Aggression score				
Tukey's test	*<0.001*	*0.04*	0.90	1.0
Cohen's *d* (95 % CI)	*1.0* (*0.41* to *1.60*)	0.5 (−0.11 to 1.05)	−0.11 (−0.70 to 0.48)	–
Index of aggression				
Tukey's test	*<0.001*	*0.02*	0.94	1.0
Cohen's *d* (95 % CI)	*0.9* (*0.30* to *1.52*)	*0.6* (*0.04* to *1.20*)	0.02 (−0.57 to 0.61)	–
Latency				
Tukey's test	*<0.001*	*0.02*	0.46	1.0
Cohen's *d* (95 % CI)	*−1.3* (*−1.97* to *−0.66*)	*−0.7* (*−1.43* to *−0.05*)	−0.3 (−1.15 to 0.43)	–
Contest score				
Tukey's test	*<0.001*	*0.05*	0.82	1.0
Cohen's *d* (95 % CI)	*0.8* (*0.24* to *1.20*)	*0.6* (*0.04* to *1.20*)	−0.23 (−0.82 to 0.35)	–

The 95 % confidence intervals for Cohen's *d* are shown. Significant results are written in italic

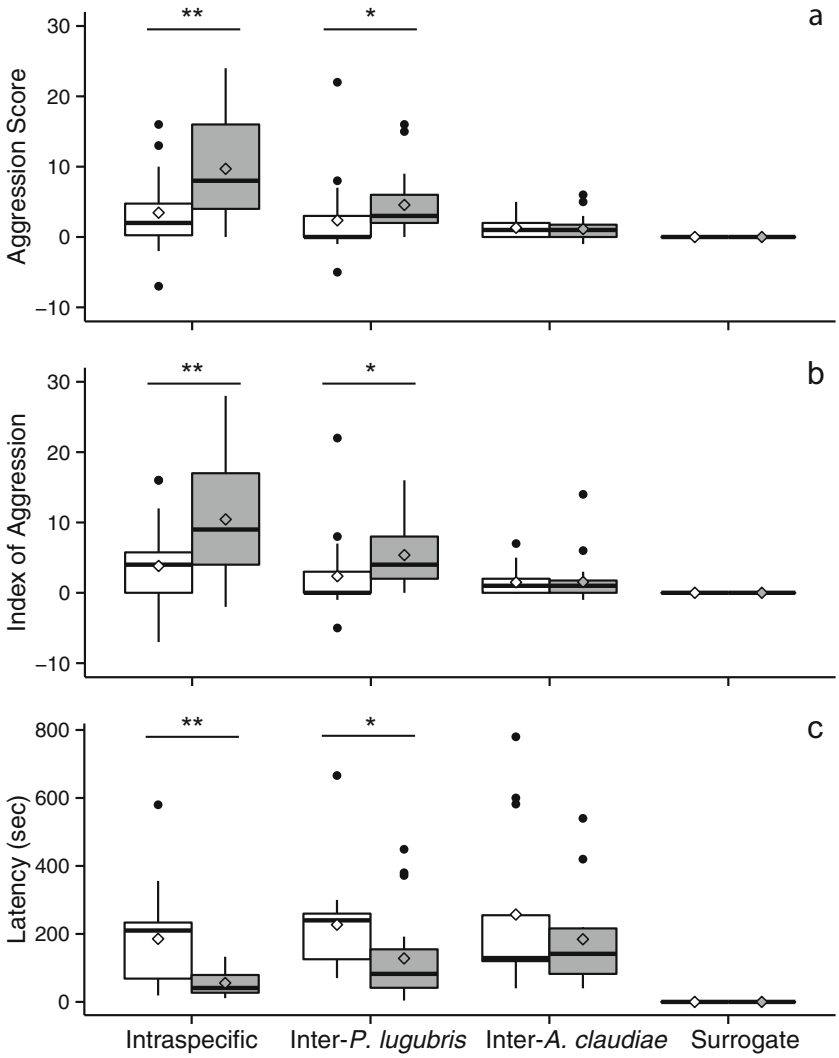

Fig. 2 Aggression levels for green (*in white*) and red (*in gray*) *Oophaga pumilio* residents paired with a conspecific (intraspecific), *Phyllobates lugubris* (interspecific *P. lugubris*), *Andinobates claudiae* (interspecific *A. claudiae*), and a surrogate (surrogate). *Positive values* represent predominance of aggressive behaviors, and *negative values* represent predominance of submissive behaviors. Three aggression metrics are shown: *a* aggression score, estimated as the number of aggressive minus submissive behaviors; *b* index of aggression, estimated as the sum of ranked values of energy expenditure of aggressive minus submissive behaviors; and *c* latency, representing time to first aggression. *Boxes* span the first and third quartiles of the data, *horizontal lines* represent the medians, and *diamond symbols* represent the means. *Whiskers* span the 95 % central ranges of the data. *Black circles* represent data outside of these ranges. Significantly different comparisons are represented by *asterisks* (*$p<0.05$; **$p<0.001$)

similar indices of aggression, as did frogs from green large-bodied and green small-bodied populations (Electronic supplementary material Table S3).

In addition, coloration interacted with treatment to explain latency (color: $F_{1,56}=11.32$, $p=0.001$; treatment: $F_{3,90}=28.36$, $p<0.001$; color×treatment: $F_{3,90}=3.13$, $p=0.03$). Tukey's post hoc tests and Cohen's d effect sizes revealed that red resident *O. pumilio* responded more quickly to same-morph conspecifics and to *P. lugubris* than green residents (Table 3, Fig. 2c). However, latency did not differ between red and green residents when paired with *A. claudiae* or with a surrogate (Table 3, Fig. 2c). Frogs from red large-bodied and red small-bodied populations exhibited similar latency, as did frogs from green large-bodied and green small-bodied populations (Electronic supplementary material Table S3).

When we assessed the effect of resident body size on their aggressive behavior, we found that body size did not influence the aggression score (size: $F_{1,57}=0.16$, $p=0.69$; size×color: $F_{1,57}=0.21$, $p=0.65$; size×treatment: $F_{3,124}=2.10$, $p=0.10$; Electronic supplementary material Fig. S2a), the index of aggression (size: $F_{1,57}=0.23$, $p=0.63$; size×color: $F_{1,57}=0.07$, $p=0.78$; size×treatment: $F_{3,124}=0.92$, $p=0.43$; Electronic supplementary material Fig. S2b), nor the latency (size: $F_{1,56}=1.18$, $p=0.28$; size×color: $F_{1,56}=0.15$, $p=0.70$; size×treatment: $F_{3,90}=2.44$, $p=0.07$; Electronic supplementary material Fig. S2c).

We also evaluated if aggressiveness of *O. pumilio* residents differed when they were exposed to same-island and different-island heterospecifics. The aggression scores and indices of aggression of residents did not differ between these two groups, although non-significant moderate to large size effect values were found in some cases (Electronic supplementary material Table S4). The latency between both groups did not differ (Electronic supplementary material Table S4).

Contest outcome

Contest score was explained by the interaction between coloration and treatment (color: $F_{1,57}=12.69$, $p<0.001$; treatment: $F_{3,125}=3.87$, $p=0.01$, color×treatment: $F_{3,125}=6.60$, $p<0.001$). Tukey's post hoc tests and Cohen's d effect sizes showed that red resident frogs exhibited higher contest scores when paired with a same-morph conspecific and marginally higher when paired with *P. lugubris* than green residents (Table 3, Fig. 3). Contest score of red residents of *O. pumilio* was significantly higher than zero when frogs were paired with same-morph conspecific intruders (Tukey's post hoc test, $p<0.001$) and with intruders of *P. lugubris* (Tukey's post hoc test, $p<0.001$), suggesting that red residents dominated most of the interactions in these two treatments. This was not the case for green residents, whose mean contest score did not significantly differ from zero in either of these two treatments (Tukey's post hoc test: intraspecific, $p=0.33$; interspecific *P. lugubris*, $p=0.24$; Fig. 3). This suggests that green *O. pumilio* did not dominate most interactions with conspecifics of the same-morph or heterospecific intruders, despite their role as resident. Red and green residents did not differ in their contest scores when paired with the heterospecific *A. claudiae* or a surrogate (Table 3, Fig. 3).

When the effect of resident body size on contest outcome was assessed, we found that body size did not influence contest score (size: $F_{1,57}=0.14$, $p=0.70$; size×color: $F_{1,57}=0.23$, $p=0.63$; size×treatment: $F_{3,125}=0.23$, $p=0.88$; Electronic supplementary material Fig. S2d).

Intruder agonistic behavior

Same-morph conspecific intruders and heterospecifics responded similarly toward resident frogs of *O. pumilio* from red and green populations (Electronic supplementary material Fig. S3). Resident coloration did not influence the intruder's aggression score (color: $F_{1,55}=3.30$, $p=0.07$; color×size: $F_{1,55}=0.44$, $p=0.51$; color×treatment: $F_{2,66}=2.47$, $p=0.09$) or intruder's index of aggression (color: $F_{1,55}=2.07$, $p=0.16$; color×size: $F_{1,55}=0.42$, $p=0.52$; color×treatment: $F_{2,66}=1.80$, $p=0.17$). Similarly, resident body size did not influence intruder's aggression score (size: $F_{1,55}=0.00$, $p=0.97$; size×color: $F_{1,55}=0.44$, $p=0.51$; size×treatment: $F_{2,66}=0.14$, $p=0.87$) or index of aggression (size: $F_{1,55}=0.02$, $p=0.89$; size×color: $F_{1,55}=0.42$, $p=0.52$; size×treatment: $F_{2,66}=0.08$, $p=0.92$).

Discussion

Our resident/intruder experiment suggests that coloration in *O. pumilio* is a good indicator of agonistic tendencies, since coloration associates with aggressiveness and the relative outcomes of agonistic intraspecific and interspecific interactions. More conspicuous red and less conspicuous green *O. pumilio* residents differed in their aggressive behavior regardless of whether they belonged to large-bodied or small-bodied populations. Residents from red populations responded quickly and exhibited a large number of aggressive behaviors against conspecifics of the same morph and the heterospecific *P. lugubris*. In contrast, residents from green populations did not respond as quickly and exhibited fewer aggressive acts under these treatments. Also, residents from red populations exhibited aggressive behaviors that were energetically expensive compared to those exhibited by residents from green populations, indicating that red frogs were likely to spend more energy than green frogs when escalating to agonistic encounters against same-morph conspecifics and *P. lugubris*. Moderate to large effect sizes (Cohen's $d\geq0.5$) for all aggression metrics indicated a strong trend for red residents, in contrast to green

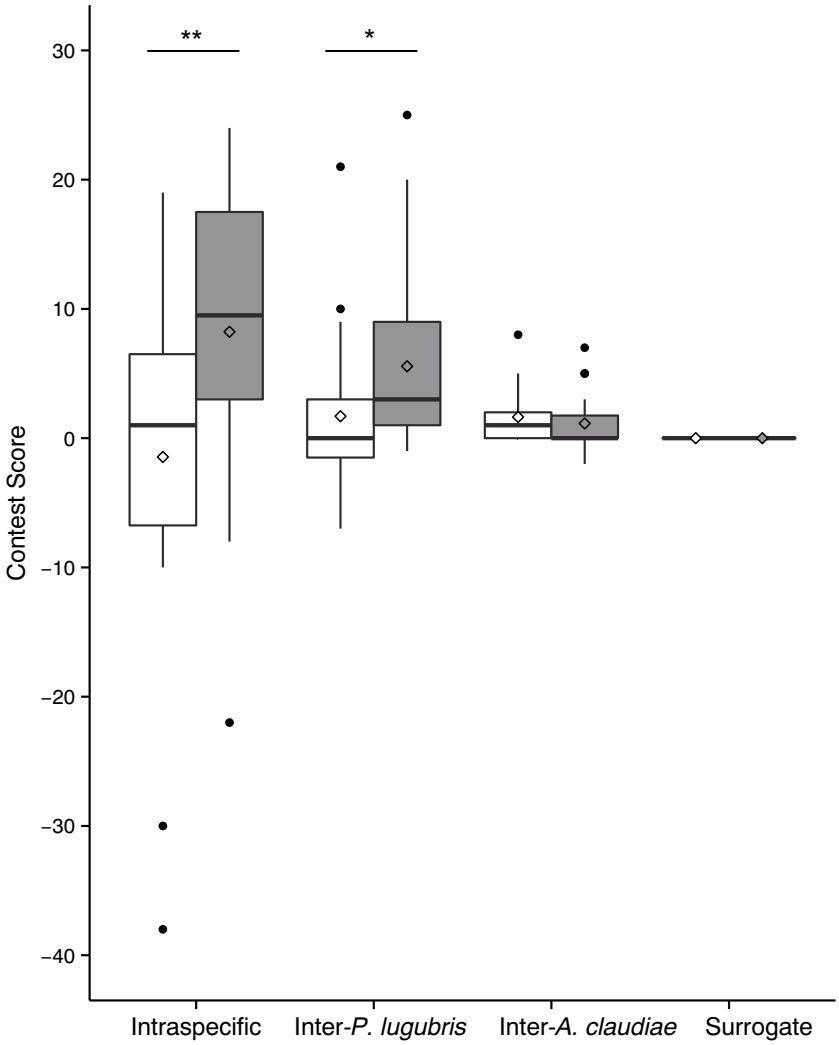

Fig. 3 Contest scores as measures of contest outcome for green (*in white*) and red (*in gray*) *O. pumilio* residents paired with a conspecific (intraspecific), *Phyllobates lugubris* (interspecific *P. lugubris*), *Andinobates claudiae* (interspecific *A. claudiae*), and a surrogate (surrogate). Increasingly, *positive values* denote dominance of the resident, and *negative values* represent dominance of the intruder. *Boxes* span the first and third quartiles of the data, *horizontal lines* represent the medians, and *diamond symbols* represent the means. *Whiskers* span the 95 % central ranges of the data. *Black circles* represent data outside of these ranges. Significantly different comparisons are represented by *asterisks* (*$p<0.05$; **$p<0.001$)

residents, to be highly aggressive against same-morph conspecifics and *P. lugubris*.

Our results are generally consistent with Rudh et al. (2013) who tested the aggressive responses of *O. pumilio* in the field using mirror-image stimulation. They observed that aggression toward population-specific conspecifics tended to be higher in populations with more conspicuous individuals, suggesting that the reduced cost of predation pressure in aposematic frogs frees them to exhibit more behaviors that affect detection, like explorative and aggressive behaviors. However, unlike Rudh et al. (2013) who measured the frequency of residents behaving aggressively, we used direct measures to quantify specific behaviors of residents and conspecifics of the same-morph or heterospecific intruder frogs. This approach allowed us to estimate variation among individuals while including the responses of intruders, which, in nature, can modulate resident behaviors and the outcomes of interactions (Peiman and Robinson 2010). Rudh et al. (2013) found aggressiveness in green individuals from the large-bodied population (Isla Colon) to be intermediate between more conspicuous and less conspicuous populations. In contrast, we found this population to exhibit similar levels of aggression to the other green population (Cayo de Agua). It is not known if the difference observed was driven by the methods used in the two studies or by the frogs tested. However, although frogs from Isla Colon are more conspicuous than frogs from other green populations and less conspicuous than red populations (Rudh et al. 2013), they likely exhibit aggressive behaviors that are as cryptic as those of very cryptic populations (this study) or intermediate between those of conspicuous populations and very cryptic populations (Rudh et al. 2013). Populations of intermediate color in another frog (*O. granulifera*) were shown to attain intermediate conspicuousness resulting from a combination of aposematic and cryptic behavioral traits (Willink et al. 2013).

Recent studies revealed conspicuous signals as good predictors of behavior in male–male interactions of an orange population of *O. pumilio*. Dorsal brightness influenced aggressive behaviors, with brighter males approaching and calling to stimulus frogs faster than duller males (Crothers et al.

2011), and being more willing to initiate aggressive interactions with conspecifics by calling more quickly than their duller counterparts (Crothers and Cummings 2015). Brightness and hue are interdependent traits of a visual signal (Maan and Cummings 2009), and *O. pumilio* from red populations included in our study exhibited brighter dorsal coloration than frogs from green populations (Maan and Cummings 2012). Therefore, our findings on high levels of aggressiveness exhibited by red frogs against conspecifics of the same morph and heterospecifics support and expand on previous studies, indicating that greater male dorsal conspicuousness (represented by hue) serves as an indicator trait in both intraspecific and interspecific behavioral encounters. We found that conspecifics of the same morph and heterospecific intruders did not respond more aggressively toward red frogs, suggesting that intruder's behavior did not drive the differences observed in the aggressive level exhibited by *O. pumilio* from red and green populations. Behavioral traits that increase detection such as aggressiveness are likely being favored in frogs from more conspicuous populations (e.g., red and orange), which are generally considered to be more toxic than frogs from green populations based on injection assays in mice (Maan and Cummings 2012; but see Daly and Myers 1967). In addition, frogs from more conspicuous populations are visually more conspicuous to predators and other frogs (Maan and Cummings 2012), yet likely suffer lower predation rates than frogs from less conspicuous populations (Hegna et al. 2013; but see Dreher et al. 2015).

Variation in the level of aggressiveness in *O. pumilio* residents of green and red populations leads to differences in dominance that likely influence their ability to secure or hold territories when encountering population-specific conspecifics or heterospecifics. Moderate to large effect sizes for contest score (Cohen's $d \geq 0.6$) indicated a strong trend for red residents to dominate encounters with same-morph conspecifics and *P. lugubris*, unlike green residents. Red frogs exhibited higher aggressive levels than same-morph conspecific and *P. lugubris* intruders, with red residents dominating 83 % of population-specific encounters and 80 % of the encounters with *P. lugubris*. Green resident frogs dominated only 54 % of population-specific encounters and 39 % of *P. lugubris* encounters, despite their status as residents. In a previous experiment with red *O. pumilio* from a red population in Costa Rica, residents consistently dominated intruders, indicating that dominance was strongly reinforced by prior residency (Baugh and Forester 1994). Our results suggest that this might be the case for frogs from red populations in the Bocas del Toro Archipelago, but not for frogs from green populations, which are less aggressive and therefore less able to dominate interactions with same-population frogs or *P. lugubris*. Aggressive males of *O. pumilio* defend territories with high densities of females, and these territories enhance male mating success (Pröhl and Berke 2001). The lower dominance in interactions by frogs from green populations could denote a lesser ability to hold high-quality territories in contrast to frogs from red populations and a limitation to establish new territories. Crothers and Cummings (2015) suggested that brighter (i.e., more conspicuous) males in one orange population of *O. pumilio* may be greater territorial threats than duller males during conspecific encounters. Our findings expand on this idea, suggesting that red population males (i.e., more conspicuous) may be better at securing and defending territories than green population males (i.e., less conspicuous) against conspecifics of the same morph and heterospecifics that may pose any threat. The effect that different levels of dominance by different morphs of *O. pumilio* in population-specific and heterospecific encounters have on the ability to hold and establish new territories deserves to be further studied. Also, studies addressing how relatively consistent outcomes of behavioral interactions—such as one morph dominating territorial interactions—translate into competitive and other types of ecological interactions in the field are relevant to improve our understanding of the ecological implications of intraspecific coloration variation.

Differences among morphs in the ability to hold a territory and differently discriminate among conspecific and heterospecific intruders are particularly interesting, because those differences may ultimately have community-level consequences for the relative abundances and distributions of other species with which *O. pumilio* interacts. For example, if the asymmetric aggressiveness between different morph populations of *O. pumilio* against heterospecifics is widespread, individuals of the dominant red morph may exclude more submissive taxa from optimal territories or resources on islands where the red morph is exclusively found. Tolerance toward heterospecifics by the less aggressive green morph, however, may allow for co-occurrence of more species or higher relative abundances on islands where this morph exclusively occurs.

A territory holder is expected to aggressively exclude a heterospecific intruder when the intruder species can be excluded at a low cost, in a short time, and when it causes the territory holder to lose fitness due to high niche overlap (Mikami and Kawata 2004). The strong aggressiveness and dominance of red *O. pumilio* toward *P. lugubris* could be the result of the low cost and short time that more conspicuous frogs spend excluding this heterospecific due to their enhanced protection from predators, which allows them to defend their territories more aggressively. However, there may also be a higher cost of *P. lugubris* intrusion for frogs from red populations. If red frogs invest more in defense mechanisms such as coloration and toxins than green frogs, for example in terms of finding suitable food (Pröhl and Ostrowski 2011), red frogs may have a higher motivation to escalate in heterospecific encounters with *P. lugubris* which could compete for food sources with *O. pumilio*

(Baugh and Forester 1994). Furthermore, *O. pumilio* also defends calling territories that are used for courtship and oviposition (Pröhl 1997). Because calls in both species have similar main frequencies, *P. lugubris* can mask the calls of *O. pumilio*, causing lowering of the dominant frequency in the latter (Wong et al. 2009). Red *O. pumilio* spend more time closer to the forest floor than green frogs (Pröhl and Ostrowski 2011). If this differential use of habitat leads to differential costs of acoustic interference by *P. lugubris* (e.g., higher costs for red frogs, which spend most of their time on the forest floor such as *P. lugubris*; Savage 2002), red frogs may be more motivated to escalate heterospecific encounters with *P. lugubris*. Therefore, a combination of the cost, time, and fitness enhancement of intruder exclusion could explain the observed behavior of *O. pumilio* toward *P. lugubris*. Conversely, the mild aggression we observed toward *A. claudiae* could be explained if this species does not pose a strong threat to *O. pumilio*, despite resource overlap (both species deposit their larvae in water-filled leave axils; Lötters et al. 2007), or if by being smaller in size, *A. claudiae* does not pose a strong threat owing to low food-resource overlap. Territorial organisms are able to adjust their aggressive responses based on the level of threats that other individuals pose to them (Lehtonen et al. 2010). The different responses of *O. pumilio* toward both heterospecifics in this study suggest that red and green phenotypes may adjust their aggressive responses according to the costs and threats that other species pose. However, studies on the adaptive nature of conspecific and heterospecific aggression are required to better understand the variation in the strength of aggression of different color morphs. Similarly, understanding the underlying mechanisms for differences in the aggression level exhibited by green and red morphs of *O. pumilio* is relevant. For example, testosterone is a recognized modulator of aggressive behavior and territoriality in birds and lizards (e.g., Sinervo et al. 2000; Pryke et al. 2007) but its effects on amphibian aggression deserves further study (Wilczynski et al. 2005). Also, differences in the social environment, such as in population or morph density, have been shown to associate with contrasting behavioral strategies in polymorphic birds (Pryke et al. 2007) and could be contributing to the patterns here observed.

Similar to coloration, body size is expected to be an important predictor for aggressiveness and dominance (Hsu et al. 2006). However, in our study, resident body size was unrelated to both conspecific and heterospecific aggressiveness. This suggests that, in *O. pumilio*, resident coloration associates with the outcome of a contest, regardless of whether the male belongs to a large-bodied or small-bodied population. This result is consistent with previous studies on a polymorphic lizard and *O. pumilio*, in which one morph dominates agonistic interactions independent of focal body size (Hover 1985; Crothers and Cummings 2015).

We showed that coloration in *O. pumilio* is a good indicator of aggressiveness, which associates with the outcome of same-morph intraspecific and some interspecific behavioral interactions, providing support for a positive relationship between the strength of anti-predator coloration, agonistic behavior, and dominance in interspecific, intraguild interactions. Even so, there are a few caveats to our conclusions. First, we challenged *O. pumilio* residents with heterospecifics from different islands, because all species did not co-occur on all islands where *O. pumilio* residents were collected. Unfamiliarity of residents with some of the intruders could have influenced our results. We did not find significant differences in the response of residents to heterospecifics from the same island or from another island. However, non-significant moderate to large effect sizes indicate that significant effects could be detected if sample sizes were to be increased. Second, we did not assess the possible effects that differences in habitat preferences by red and green *O. pumilio* may have on their level of aggressiveness. For example, differences in the amount of time that frogs of different morphs spend on the forest floor (Pröhl and Ostrowski 2011), or at different perch heights (Dugas et al. 2015), could increase the rate at which they encounter other frogs or their propensity to escalate to agonistic interactions. Third, our results are based on the study of two red and two green populations. Because behavioral traits are more prone to evolutionary changes than morphological traits (Blomberg et al. 2003), it would be interesting to assess this association in other populations of the species, including polymorphic populations. Particularly, studies including encounters between cryptic and conspicuous morphs would improve our understanding of the association between coloration and conspecific aggressiveness and the evolution of divergent coloration.

Red and orange colorations associate with aggressiveness and dominance in conspecific agonistic encounters in several taxa (Evans and Norris 1996; Sinervo et al. 2000; Pryke and Griffith 2006; Healey et al. 2007). Our study shows that this association also functions in the context of interspecific interactions, suggesting that conspicuous traits that likely have been shaped by natural selection can function not only as honest intraspecific sexual communication signals (Crothers and Cummings 2015) but also as honest interspecific communication signals. In an example similar to ours, Dijkstra et al. (2005) found red male *Pundamilia* fishes to dominate blue males of their same species and other congeners in dyadic combats. Dominance of red males corresponded with the geographic distribution of both morphs (red and blue morphs co-occurred in some populations, and blue populations were also common), suggesting that the dominance advantage of red morphs facilitated the invasion of blue populations (Dijkstra and Groothuis 2011). More empirical investigations are needed in multiple taxa to better understand whether the increased aggression of red individuals toward heterospecifics is

widespread, the role that this relationship plays in interspecific conflict resolution, and the possible evolutionary and ecological consequences of this behavior-coloration association.

Understanding the asymmetric dominance of morphs, as in our study (i.e., when one color morph dominates encounters with conspecifics and heterospecifics), can improve our grasp of both the evolutionary (e.g., diversification) and ecological (e.g., species co-occurrence) consequences of the association between coloration and agonistic behavior. In the case of evolutionary consequences, sexual selection can influence the direction of aposematic trait evolution, because signals shaped by natural selection also function in mate selection (Crothers and Cummings 2015). However, heterospecific aggression also has been shown to be adaptive and influence signal evolution (Adams 2004; Peiman and Robinson 2007; Laiolo 2012). Just as individual traits should be examined as components of integrated phenotypes, individual selective influence (e.g., mate choice, heterospecific interactions) integrates to form an organism's overall selective environment. If aposematism confers additional benefits to protection from predators, such as access to more resources as a result of dominant advantage not only over conspecifics but also over competing heterospecifics, these benefits could likely influence character diversification (Speed et al. 2010). In the case of ecological consequences, negative interactions such as heterospecific aggression, can affect resource and habitat use and may therefore influence community structure (reviewed by Peiman and Robinson 2010). If aggression levels associate with presumed signaling traits such as coloration, the latter can influence the abundance and distribution of individuals and species at the community scale. For example, coexistence of species was shown to be facilitated by color differences associated with aggressiveness in cichlid fishes in Lake Victoria, where negative frequency-dependent selection on color among closely related species affected species distributions (Seehausen and Schluter 2004). Ongoing research comparing the results of this study with the abundances and distributions of species will further reveal the interplay among intraspecific coloration and behavioral variation, species interactions, and community structure. This approach would add to our understanding of the potential for intraspecific traits shaped by natural and sexual selection to impact species interactions and ultimately influence ecological patterns and processes at the community level.

Acknowledgments We are grateful to M. Caldwell, J. Cronin, J. Davis, B. Elderd, C. Richards-Zawacki, and R. Stevens for their helpful suggestions during the development and execution of this study. Many thanks to C. Richards-Zawacki for providing equipment and to L. Freeborn for providing information about frog localities. For their assistance in the field, we thank D. Gonzales, C. Harris, M. Alvarez, and A. Cuervo. The Smithsonian Tropical Research Institute provided logistical support, and we especially thank G. Jacome, P. Gondola, C. Jaramillo, and the Bocas del Toro Research Station staff. We thank M. Dugas, K. Hovanes, M. Klock, J. Touchon, A. Stuckert, K. Summers, members of the Richards-Zawaki Lab, and two anonymous reviewers for their comments on drafts of the manuscript and D. Blouin for providing statistical advice. This study was supported by the Sigma Xi Chapter of Louisiana State University and by two grants from the Louisiana Environmental and Education Commission from the Louisiana Department of Wildlife and Fisheries.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. Experiments reported in this article comply with the current laws of Panama (ANAM permit No. SC/A-41-12). All procedures performed were in accordance with the ethical standards of Louisiana State University (IACUC No. 13-006) and the Smithsonian Tropical Research Institute (IACUC No. 2012-1015-2015).

References

Adams DC (2004) Character displacement via aggressive interference in Appalachian salamanders. Ecology 85:2664–2670

Anderson CN, Grether GF (2010) Interspecific aggression and character displacement of competitor recognition in *Hetaerina* damselflies. Proc R Soc Lond B 277:549–555

Bastiaans E, Morinaga G, Gaytan JGC, Marshall JC, Sinervo B (2013) Male aggression varies with throat color in 2 distinct populations of the mesquite lizard. Behav Ecol 24:968–981

Baugh JR, Forester DC (1994) Prior residence effect in the dart-poison frog, *Dendrobates pumilio*. Behaviour 131:207–224

Bee MA (2003) A test of the "dear enemy effect" in the strawberry dart-poison frog (*Dendrobates pumilio*). Behav Ecol Sociobiol 54:601–610

Bernays EA, Singer M (2002) Contrasted foraging tactics in two species of polyphagous caterpillars. Acta Zool Hung 48:117–135

Blomberg SP, Garland T Jr, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717–45

Brown JL, Maan ME, Cummings ME, Summers K (2010) Evidence for selection on coloration in a Panamanian poison frog: a coalescent-based approach. J Biogeogr 37:891–901

Cohen J (1988) Statistical power analyses for the behavioral sciences, 2nd edn. Erlbaum, Hillsdale

Crothers LR, Cummings ME (2013) Warning signal brightness variation: sexual selection may work under the radar of natural selection in populations of a polytypic poison frog. Am Nat 181:E116–E124

Crothers LR, Cummings ME (2015) A multifunctional warning signal behaves as an agonistic status signal in a poison frog. Behav Ecol 26:560–568

Crothers LR, Gering E, Cummings ME (2011) Aposematic signal variation predicts male-male interactions in a polymorphic poison frog. Evolution 65:599–605

Daly JW, Myers CW (1967) Toxicity of Panamanian poison frogs (*Dendrobates*): some biological and chemical aspects. Science 156:970–973

Dijkstra PD, Groothuis TG (2011) Male-male competition as a force in evolutionary diversification: evidence in haplochromine cichlid fish. Int J Evol Biol 2011:689254

Dijkstra PD, Seehausen O, Groothuis TGG (2005) Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. Behav Ecol Sociobiol 58:136–143

Dijkstra PD, van Dijk S, Groothuis TGG, Pierotti MER, Seehausen O (2009) Behavioral dominance between female color morphs of a Lake Victoria cichlid fish. Behav Ecol 20:593–600

Donnelly MA (1989) Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. Ecol Monogr 59:207–221

Dreher CE, Cummings ME, Proehl H (2015) An analysis of predator selection to affect aposematic coloration in a poison frog species. PLoS One 10, e0130571

Duckworth RA, Badyaev AV (2007) Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. Proc Natl Acad Sci U S A 104:15017–15022

Dugas MB, Halbrook SR, Killius AM, Sol JF, Richards-Zawacki CL (2015) Colour and escape behaviour in polymorphic populations of an aposematic poison frog. Ethology 121:813–822

Evans MR, Norris K (1996) The importance of carotenoids in signalling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). Behav Ecol 7:1–6

Forester D, Cover J, Wisnieski A (1993) The influence of time of residency on the tenacity of territorial defense by the dart-poison frog *Dendrobates pumilio*. Herpetologica 49:94–99

Gehara M, Summers K, Brown JL (2013) Population expansion, isolation and selection: novel insights on the evolution of color diversity in the strawberry poison frog. Evol Ecol 27:797–824

Grant JB (2007) Ontogenetic colour change and the evolution of aposematism: a case study in panic moth caterpillars. J Anim Ecol 76:439–447

Grether GF, Anderson CN, Drury JP, Kirschel ANG, Losin N, Okamoto K, Peiman KS (2013) The evolutionary consequences of interspecific aggression. Ann N Y Acad Sci 1289:48–68

Healey M, Uller T, Olsson M (2007) Seeing red: morph-specific contest success and survival rates in a colour-polymorphic agamid lizard. Anim Behav 74:337–341

Hegna RH, Saporito RA, Donnelly MA (2013) Not all colors are equal: predation and color polytypism in the aposematic poison frog *Oophaga pumilio*. Evol Ecol 27:831–845

Heying H (2004) Reproductive limitation by oviposition site in a treehole breeding Madagascan poison frog (*Mantella laevigata*). Misc Publ Mus Zool Univ Mich 193:23–30

Hödl W, Amézquita A (2001) Visual signaling in anuran amphibians. In: Ryan MJ (ed) Anuran communication. Smithsonian Institute Press, Washington, pp 121–141

Hover EL (1985) Differences in aggressive-behavior between two throat color morphs in a lizard, *Urosaurus ornatus*. Copeia 1985:933–940

Hsu Y, Earley RL, Wolf LL (2006) Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. Biol Rev Camb Philos 81:33–74

Kenward MG, Roger JH (1997) Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983–997

Laiolo P (2012) Interspecific interactions drive cultural co-evolution and acoustic convergence in syntopic species. J Anim Ecol 81:594–604

Lehtonen TK, McCrary JK, Meyer A (2010) Territorial aggression can be sensitive to the status of heterospecific intruders. Behav Process 84: 598–601

Lieberman SS (1986) Ecology of the leaf litter herpetofauna of a neotropical rain forest: La Selva, Costa Rica. Acta Zool Mex 15:1–72

Lötters S, Jungfer KH, Henkel FW, Schmidt W (2007) Poison frogs, biology, species & captive husbandry. In: Edition Chimaira & Serpents Tale. Frankfurt am Main, Germany

Maan ME, Cummings ME (2008) Female preferences for aposematic signal components in a polymorphic poison frog. Evolution 62: 2334–2345

Maan ME, Cummings ME (2009) Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. Proc Natl Acad Sci U S A 106:19072–19077

Maan ME, Cummings ME (2012) Poison frog colors are honest signals of toxicity, particularly for bird predators. Am Nat 179:E1–E14

Merilaita S, Tullberg BS (2005) Constrained camouflage facilitates the evolution of conspicuous warning coloration. Evolution 59:38–45

Mikami OK, Kawata M (2004) Does interspecific territoriality reflect the intensity of ecological interactions? A theoretical model for interspecific territoriality. Evol Ecol Res 6:765–775

Mochida K (2009) A parallel geographical mosaic of morphological and behavioural aposematic traits of the newt, *Cynops pyrrhogaster* (Urodela: Salamandridae). Biol J Linn Soc 97:613–622

Peiman KS, Robinson BW (2007) Heterospecific aggression and adaptive divergence in brook stickleback (*Culaea inconstans*). Evolution 61:1327–1338

Peiman KS, Robinson BW (2010) Ecology and evolution of resource-related heterospecific aggression. Q Rev Biol 85:133–158

Pigliucci M (2003) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecol Lett 6:265–272

Pröhl H (1997) Territorial behaviour of the strawberry poison-dart frog, *Dendrobates pumilio*. Amphibia-Reptilia 18:437–442

Pröhl H, Berke O (2001) Spatial distributions of male and female strawberry poison frogs and their relation to female reproductive resources. Oecologia 129:534–542

Pröhl H, Hagemann S, Karsch J, Höbel G (2007) Geographic variation in male sexual signals in strawberry poison frogs (*Dendrobates pumilio*). Ethology 113:825–837

Pröhl H, Ostrowski T (2011) Behavioural elements reflect phenotypic colour divergence in a poison frog. Evol Ecol 25:993–1015

Prudic KL, Skemp AK, Papaj DR (2007) Aposematic coloration, luminance contrast, and the benefits of conspicuousness. Behav Ecol 18: 41–46

Pryke SR, Astheimer LB, Buttemer WA, Griffith SC (2007) Frequency-dependent physiological trade-offs between competing colour morphs. Biol Lett 3:494–497

Pryke SR, Griffith SC (2006) Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. Proc R Soc Lond B 273:949–957

Reynolds RG, Fitzpatrick BM (2007) Assortative mating in poison-dart frogs based on an ecologically important trait. Evolution 61:2253–2259

Richards-Zawacki CL, Cummings ME (2010) Intraspecific reproductive character displacement in a polymorphic poison dart frog, *Dendrobates pumilio*. Evolution 65:259–267

Robinson SK, Terborgh J (1995) Interspecific aggression and habitat selection by Amazonian birds. J Anim Ecol 64:1–11

Rojas B, Devillechabrolle J, Endler JA (2014) Paradox lost: variable colour-pattern geometry is associated with differences in movement in aposematic frogs. Biol Lett 10:20140193

Rudh A, Breed MF, Qvarnstrom A (2013) Does aggression and explorative behaviour decrease with lost warning coloration? Biol J Linn Soc 108:116–126

Rudh A, Rogell B, Hastad O, Qvarnstrom A (2011) Rapid population divergence linked with co-variation between coloration and sexual display in strawberry poison frogs. Evolution 65:1271–1282

Rudh A, Rogell B, Hoglund J (2007) Non-gradual variation in colour morphs of the strawberry poison frog *Dendrobates pumilio*: genetic and geographical isolation suggest a role for selection in maintaining polymorphism. Mol Ecol 16:4284–4294

Sacchi R, Pupin F, Gentilli A, Rubolini D, Scali S, Fasola M, Galeotti P (2009) Male-male combats in a polymorphic lizard: residency and size, but not color, affect fighting rules and contest outcome. Aggress Behav 35:274–283

SAS Institute I (2003) SAS/STAT user's guide Release 91 SAS Institute. Cary, North Carolina

Savage JM (2002) The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas. University of Chicago Press, Chicago

Seehausen O, Schluter D (2004) Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. Proc R Soc Lond B 271:1345–1353

Siddiqi A, Cronin TW, Loew ER, Vorobyev M, Summers K (2004) Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. J Exp Biol 207:2471–2485

Sinervo B, Lively CM (1996) The rock-paper-scissors game and the evolution of alternative male strategies. Nature 380:240–243

Sinervo B, Miles DB, Frankino WA, Klukowski M, DeNardo DF (2000) Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. Horm Behav 38:222–233

Speed MP, Brockhurst MA, Ruxton GD (2010) The dual benefits of aposematism: predator avoidance and enhanced resource collection. Evolution 64:1622–1633

Stamp NE, Wilkens RT (1993) On the cryptic side of life: being unapparent to enemies and the consequences for foraging and growth of caterpillars. In: Caterpillars: ecological and evolutionary constraints on foraging. Chapman & Hall, New York, pp 283–330

Summers K, Symula R, Clough M, Cronin T (1999) Visual mate choice in poison frogs. Proc R Soc Lond B 266:2141–2145

Tazzyman SJ, Iwasa Y (2010) Sexual selection can increase the effect of random genetic drift—a quantitative genetic model of polymorphism in *Oophaga pumilio*, the strawberry poison-dart frog. Evolution 64:1719–1728

Toft CA (1980) Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. Oecologia 45:131–141

Wang IJ, Shaffer HB (2008) Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. Evolution 62:2742–2759

Wilczynski W, Lynch KS, O'Bryant EL (2005) Current research in amphibians: studies integrating endocrinology, behavior, and neurobiology. Horm Behav 48:440–450

Willink B, Brenes-Mora E, Bolanos F, Proehl H (2013) Not everything is black and white: color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. Evolution 67:2783–2794

Wong S, Parada H, Narins PM (2009) Heterospecific acoustic interference: effects on calling in the frog *Oophaga pumilio* in Nicaragua. Biotropica 41:74–80